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Dated 21 June 1999.

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Request for grant of a patent

(See notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

1. Your reference — P21828/CPA/RMC — 23 MAY 1998

2. Patent application number *(The Patent Office will fill in this part)* — 23 MAY 1998 — 9811103.2

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

   Danstar Ferment A.G.
   Eden Valley Works
   Eden Valley Row
   Freuchie
   FIFE KY15 7AE

   Patents ADP number *(if you know it)* — 7442718001

   If the applicant is a corporate body, give the country/state of its incorporation — UK

4. Title of the invention — "Yeast"

5. Name of your agent *(if you have one)*

   Murgitroyd & Company

   "Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

   373 Scotland Street
   GLASGOW
   G5 8QA

   Patents ADP number *(if you know it)* — 1198013

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

   a) any applicant named in part 3 is not an inventor, or
   b) there is an inventor who is not named as an applicant, or
   c) any named applicant is a corporate body.
   See note (d))

   Yes

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9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	8
Claim(s)	3
Abstract	
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10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature Murgitroyd & Company Date 22 May 1998
Murgitroyd & Company

12. Name and daytime telephone number of person to contact in the United Kingdom: Roisin McNally, 0141 307 8400

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Notes

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"Yeast"

The present invention relates to the supply of essential minerals during alcoholic fermentations.

Zinc is a metalloenzyme and an essential mineral for yeast.

There has been an increasing awareness of the importance of zinc in alcoholic fermentations, particularly with respect to beer. Lack of sufficient zinc in beer fermentations is characterised by slow or sluggish fermentations, poor attenuation and low yeast crop.

It is thought now that beer worts were always intrinsically short of zinc but this was masked because of the nature of the construction materials that were used traditionally in the brewing plant. Gradually and eventually all copper vessels, pipes, and equipment as well as galvanised steel, have been replaced with stainless steel. Beer yeasts are relatively immune to the toxic effects of copper, unlike wine yeasts, and it is thought that as the copper slowly dissolved or was abraded into the beer wort, zinc would be so too.

The raw materials used in brewing, particularly malted barley and hops, are rich in zinc but it is bound up and does not become available to the yeast during conventional brewing processes.

Attempts by breweries to release this zinc have not been successful. These consisted of acidic extraction but along with the zinc came all manner of odiferous and haze forming moieties that rendered the beer undrinkable.

Zinc is now added, in the form of zinc sulphate, by most breweries in the world up to a level of about 0.2 ppm of wort. This generally gives an intracellular concentration of 45-55 µg/g dry yeast. Experts believe that an intracellular concentration of 100 mg/Kg dry yeast (equivalent to 100 µg/g dry yeast) is necessary and modern brewing equipment and techniques lead to beer worts with sub optimal zinc.

At the moment in the UK breweries have the choice of labelling all their ingredients or not labelling. This assumes that the raw materials used are wholesome and permitted. It is believed that this will change in the near future and full label declaration will be mandatory across Europe. In that situation the brewers may be required to put zinc, with its E number on the label. At the moment brewers argue that zinc does not pass into the beer but is consumed by the yeast and thus is a "process aid". Like diatomaceous earth, zinc need not be declared as a raw material. However by this interpretation yeast is not a raw material because it too does not pass out into the final beer. There is y ast in the final product, either by design or by omission of removal. Thus zinc is very definitely a raw material.

Some breweries now add zinc sulphate directly to the wort at the boiling stage.

However no food grade zinc salt exists in Europe. Pharmaceutical grade and chemical grade both exist. Some brewers use pharmaceutical grade, at about £12.00 per Kilo whilst others buy chemical grade, via an intermediary at about £3.00.

In Germany, because of the Reinheitsgebot (German beer purity laws) it is forbidden to use anything in the production of beer except malted barley or wheat for wheat beer, water hops and yeast. These raw materials are not defined. The Rheinheitsgebot is not a legal requirement under European law and thus German brewers can brew beer with added "chemicals" but it must be for export only. Similarly brewers outside Germany need not conform to the Reinheitsgebot for beer sold into Germany.

However the Reinheitsgebot is observed in Germany and is promoted positively as a guarantee of wholesomeness and goodness.

Thus the Germans have real problems in addressing zinc deficiency. They are reputed to suspend zinc sheets in the lactic souring vessels. It works but cannot be controlled. Zinc surplus also can cause problems with the yeast.

It is an object of the present invention to provide yeast enriched with zinc which could qualify as being yeast and addition of this would not offend the Reinheitsgebot. The zinc would have to be intracellular; that is to say it would have to be demonstrated conclusiv ly, not to have been added

extraneously.

It is another object to be able to supply essential minerals in minute quantities to the yeasts carrying out alcoholic fermentations, usually Saccharomyces cerevisiae species.

Yeast has an exceptional ability to absorb heavy metals. This means that it can be used to detoxify effluents of radio-activity as well as mercury etc.

The present invention aims to use this accumulative effect within the cell so as to concentrate essential minerals. This yeast would then be used as a sacrificial additive to certain alcoholic fermentations or it can be on-processed to make a yeast extract or yeast fractions that can also be used as a yeast trace nutrient.

According to the present invention there is provided mineral enriched yeast suitable for use in fermentation processes.

Preferably the mineral enriched yeast is zinc enriched yeast.

Alternatively the mineral enriched yeast may be manganese or magnesium enriched yeast.

The invention also provides use of zinc enriched yeast in alcoholic fermentations.

The invention also provides an alcoholic fermentation process including the step of adding mineral enriched yeast to wort.

Preferably the yeast is zinc enriched yeast.

Preferably the yeast is adding to boiling wort.

Preferably frozen mineral enriched yeast is added to boiling wort to ensure that the zinc is released.

Typically the yeast comprises 50-150 mg zinc/kg dry yeast.

The invention also provides a method for preparing zinc enriched yeast the comprising adding 0.02 to 20 ppm zinc salt to a live culture of yeast at a temperature of 25-35°C at a pH of between 3.5 to 7.0 for between 1 to 20 hours.

Example

Production of Sacrificial Zinc Yeast

Zinc, at a concentration of between 0.02 and 20 ppm, in the form of zinc sulphate, chloride or phosphate but preferably sulphate is added to a live culture of S. cerevisiae at a temperature of 25-35°C, preferably 25-32°C, at a pH of between 3.5 to 7.0, preferably 4.6-5.5, for a period if 1-20 hours, preferably 2-16 hours to allow the yeast culture to incorporate the zinc. The yeast culture may or may not be dried and be in a live or inactive form and may be whole, intact or further processed and fractionated.

The method can use a variety of zinc salts and the final condition of the yeast could be whole, live or dead, dried or not, intact, ruptured, homogenised or fractionated.

When releasing zinc from yeast during a f rmentation process it may be necessary to add acid or salt to rupture yeast cells. This may not be desirable as it could be contrary to purity laws. In circumstances where yeast will not rupture on boiling it may be advisable to freeze wet zinc loaded yeast and add yeast to boiling mixture in frozen state.

Zinc loaded yeast may be supplied in frozen state or dried to be rehydrated and frozen prior to use.

Yeast may be living or dead.

There are a number of minerals that are required in trace amounts for efficient alcoholic fermentation by yeast. These include, but not exhaustively so, manganese, magnesium and zinc. These minerals would be accumulated by the yeast and stored or contained within the cell. The exact storage point and mechanism are unclear. Some zinc, for example, may be stored in a state chemically or biologically combined within the cell and some may just be accumulated as the free ion within the cell.

It may be possible that such minerals may be manipulated during the propagation stage to produce commercially supplied yeast in dried or other preserved form that would have some desired performance.

For example a yeast loaded with zinc would tend to produce more yeast cells during the alcoholic fermentation. This could reduce the concentration of alcohol produced, which in some circumstances could be desired. Such yeasts would tend to become less robust by the end of fermentation and lyse (degenerate) rapidly. This could be advantageous in some

circumstances wh n an acceleration of maturation is d sired. The maturation of certain wines and styles can be linked to yeast autolysis. Moreover the yeast growth enhancement brought about by extra zinc can be stabilised by the use of manganese which is known to moderate the fragility factors already mentioned. Thus the autolysis factor may be modified or controlled by manipulating the intracellular concentrations of certain minerals.

Magnesium is reported to be important for alcohol efficiency in fermentations. There is a problem in the fermentation of certain substrates where there is an excess of calcium ions present. Calcium is known to be antagonistic to magnesium metabolism and in beer for example calcium is deliberately added in order to control the pH (acidity) and activate some of the enzymes of the malted barley. It may be possible to load the yeast cell up with magnesium before it is pitched into the wort so as to negate the repressive affect of the calcium and thereby increase sugar/alcohol conversion.

Finally there is the possibility of using yeast that has been pre-loaded with excess amounts of minerals as sacrificial yeast nutrient. This could be in the form of a pre-prepared yeast extract or yeast fraction. Such a preparation would provide a readily assimilable nutrient for the fermentation yeast. It is possible that the minerals associated with these preparations may be in a form that can be more readily assimilated than the native chemical mineral. It is possible that some such preparations would legally be described as yeast extracts and would be permitted where the use of the raw mineral was not. Such an example is that of magnesium which, although not banned, is not

specifically permitted for historical or obscure reasons.

Perhaps the most interesting use of sacrificial mineral enriched yeasts lies within the German brewing industry. There, only yeast, hops, malted barley or wheat and water may be used for the production of beer. Zinc is now known to be limiting in German modern beer fermentations because in olden times copper was used as tank and pipe fabrication metal. Zinc would leech from the copper and anodised steel. Stainless steel does not have this as a constituent. Forbidden by the "Reihnheitsgebot" or German purity laws from adding mineral zinc or zinc salts as a raw material there is a real problem. Such a zinc enriched living yeast could be added directly to the wort such that it is killed during the wort boiling stage. The zinc would then pass into the wort and be assimilated by the yeast during the alcoholic fermentation stage thereby eliminating the deficiency. Alternatively a yeast extract could be made of the zinc enriched yeast and added directly to the fermenter, yeast holding vessel or even the wort boiling vessel to achieve the same end as the added whole yeast cells.